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MINIREVIEW

Carbapenemase-Producing *Klebsiella pneumoniae*, a Key Pathogen Set for Global Nosocomial Dominance

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The management of infections due to *Klebsiella pneumoniae* has been complicated by the emergence of antimicrobial resistance, especially to carbapenems. Resistance to carbapenems in *K. pneumoniae* involves multiple mechanisms, including the production of carbapenemases (e.g., KPC, NDM, VIM, OXA-48-like), as well as alterations in outer membrane permeability mediated by the loss of porins and the upregulation of efflux systems. The latter two mechanisms are often combined with high levels of other types of β-lactamases (e.g., AmpC). *K. pneumoniae* sequence type 258 (ST258) emerged during the early to mid-2000s as an important human pathogen and has spread extensively throughout the world. ST258 comprises two distinct lineages, namely, clades I and II, and it seems that ST258 is a hybrid clone that was created by a large recombination event between ST11 and ST442. Incompatibility group F plasmids with bla_{KPC} have contributed significantly to the success of ST258. The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* remains unknown. Some newer agents show promise for treating infections due to KPC producers; however, effective options for the treatment of NDM producers remain elusive.

The genus *Klebsiella* belongs to the family *Enterobacteriaceae*, which includes saprophytes often isolated from the environment. *Klebsiella pneumoniae* is the most clinically relevant *Klebsiella* species and is responsible for over 70% of human infections due to this genus (1). In humans, *K. pneumoniae* most often colonizes the gastrointestinal tract, skin, and nasopharynx and is an important cause of serious community onset infections such as necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis (2, 3). During the 1970s, *K. pneumoniae* became an important cause of nosocomial infections, especially urinary tract infections (UTIs), respiratory tract infections, and bloodstream-associated infections (BSIs) (1, 2, 4). A recent report from the CANWARD surveillance program showed that *K. pneumoniae* was the fifth most common bacterium isolated in Canadian hospitals from 2007 to 2011 (5).

The management of infections due to *K. pneumoniae* has been complicated by the emergence of antimicrobial resistance, especially since the 1980s. The cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are often used to treat infections due to *K. pneumoniae*, and resistance to these agents generates delays in appropriate empirical therapy with subsequent increased morbidity and mortality in patients (6). Therefore, clinical therapeutic choices for treating nosocomial infections due to *K. pneumoniae* have become challenging (6–8). Several global surveillance studies during the 2000s have shown that 20 to 80% of *K. pneumoniae* isolates were resistant to first-line antibiotics, including the cephalosporins, fluoroquinolones, and aminoglycosides (9–11). Of special concern is the emerging resistance to carbapenems, since these agents are often the last line of effective therapy available for the treatment of infections caused by multidrug-resistant (MDR) *K. pneumoniae* (12).

Recently, the World Health Organization (WHO) released a report entitled *Antimicrobial resistance: global report on surveillance 2014* (13), which focused on antibiotic resistance in seven different bacteria responsible for common serious diseases such as bloodstream infections, diarrhea, pneumonia, UTIs, and gonorrhea. Specifically for *K. pneumoniae*, the WHO report (13) concluded that resistance to the treatment of last resort for life-threatening infections caused by a common intestinal bacterium, *K. pneumoniae*, i.e., carbapenem antibiotics, has spread to all regions of the world. *K. pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, and infections in newborns and intensive care unit patients. In some countries, because of resistance, carbapenem antibiotics would not work in more than half of the people treated for *K. pneumoniae* infections.

The aim of this article is to provide a brief overview of the mechanisms responsible for carbapenem resistance in this species, highlighting recent developments in the clonal expansion of certain high-risk sequence types (STs) or clones, and describe the role of epidemic plasmids in the global dissemination and success of carbapenem-resistant *K. pneumoniae*. Sections on virulence and treatment are also included.

MECHANISMS OF RESISTANCE TO CARBAPENEMS

Resistance to carbapenems in *K. pneumoniae* is linked to different mechanisms (14). The co-occurrence of permeability defects, together with the production of β-lactamases that possess very weak carbapenemase activity, may lead to reduced susceptibility to carbapenems, particular ertapenem (15). Such enzymes may be either Ambler class A extended-spectrum β-lactamases (ESBLs) or Ambler class C AmpC cephalosporinases, and some of them

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TABLE 1 Characteristics of *K. pneumoniae* strains that produce carbapenemases

Enzyme types (class) and examples	Spectrum of activity	Inhibitor(s)	Areas of endemicity	Molecular epidemiology
MBLs (B): NDM-1, IMP, VIM	Penicillins, cephalosporins, cephamycins, carbapenems	Metal chelators, e.g., EDTA, dipicolinic acid	Japan (IMP), Taiwan (IMP), Indian subcontinent (NDM), Balkan states (NDM), Greece (VIM)	IncA/C, N plasmids (NDM), class I integrons (VIM, IMP)
KPCs (A): KPC-2, -3, others	Penicillins, cephalosporins, cephamycins, carbapenems	Clavulanic acid (weak), tazobactam(weak), boronic acid, avibactam	United States, Greece, Italy, Israel, China, Brazil, Colombia, Argentina	Tn*4401*, IncFII plasmids, CC258
OXA-β-lactamases (D): OXA-48, OXA-181, OXA-204, OXA-232	Penicillins, temocillin, β-lactamase inhibitor combinations, carbapenems (weak)	NaCl	Turkey, North Africa (Morocco, Tunisia), Europe (Spain, Belgium)	Tn*1999*, IncL/M plasmids

(i.e., CTX-M-15, CMY-2) are more likely to contribute to reduced carbapenem susceptibility when combined with permeability defects (16).

Apart from those mechanisms involving β-lactamases (e.g., ESBLs, AmpC), which are not considered significant carbapenem-hydrolyzing enzymes, true carbapenemases are responsible for nonsusceptibility to carbapenems without additional permeability defects in *K. pneumoniae*. Those carbapenemases belong to Ambler molecular class A, B, or D (17).

The class A KPC-type β-lactamases have been extensively and almost exclusively reported in *K. pneumoniae* (18). KPC-1 (which was later shown to be identical to KPC-2) was reported in the late 1990s in a *K. pneumoniae* isolate in North Carolina. To date, more than 20 different KPC variants have been described, even though KPC-2 and -3 remain the most commonly identified variants (19). These enzymes provide resistance to the penicillins, carbapenems, cephalosporins, cephamycins, and monobactams and are inhibited by β-lactamase inhibitors such as clavulanic acid (weakly), tazobactam (weakly), boronic acid, and avibactam. KPC β-lactamases (especially KPC-2 and -3) have been described in several enterobacterial species, especially *Klebsiella* spp. and to a lesser extent in *Enterobacter* spp. (20). Several nosocomial outbreaks, most often due to *K. pneumoniae*, have been reported in North America (especially the United States), South America (Colombia, Argentina), Europe (Greece, Italy, Poland), Asia (China), and the Middle East (Israel) (19, 21, 22). KPC-producing bacteria are considered to be endemic in certain parts of the world, such as the northeastern United States, Puerto Rico, Colombia, Greece, Italy, Israel, and China, and are important causes of nosocomially acquired infections in some parts of these countries (22). *K. pneumoniae* ST258 with KPC-2 and KPC-3 has contributed significantly to the worldwide distribution of this resistance trait (more details are provided in the high-risk clone section) (22). In addition, there are some scattered reports of GES-5, another class A carbapenemase that is a point mutant derivative of GES-1 (23).

The class B β-lactamases or metallo-β-lactamases (MBLs) identified in *K. pneumoniae* have also been identified in various enterobacterial species (17). They are mainly NDM-, VIM-, and IMP-type enzymes, with the first group being the most commonly identified worldwide. Although IMP producers are identified mainly in China, Japan, and Australia, VIM-producing *K. pneumoniae* isolates are found mainly in Italy and Greece (17). NDM-1 shares very little identity with other MBLs, the most similar being VIM-1/VIM-2, with only 32.4% amino acid identity. Since the first description of NDM-1, more than 10 variants of this enzyme have been described, the majority of them originated in Asia (24). Bacteria with MBLs are often resistant to penicillins, carbapenems, cephalosporins, and cephamycins but remain susceptible to monobactams, and their activity is inhibited by metal chelators such as EDTA and dipicolinic acid (Table 1). The majority of NDM-1-producing bacteria also carry a diversity of other resistance mechanisms (17). These additional mechanisms include the following: plasmid-mediated AmpC β-lactamases (especially CMY types), ESBLs (especially CTX-M-15), different carbapenemases (e.g., OXA-48, VIM, and KPC types), 16S rRNA methyltransferases, plasmid-mediated quinolone resistance determinants, macrolide-modifying esterases, and rifampin-modifying enzymes. Consequently, *Enterobacteriaceae* with NDM-type enzymes remain mostly susceptible to agents such colistin, fosfomycin, and tigecycline (24).

The only class D carbapenem-hydrolyzing β-lactamase found in *K. pneumoniae* isolates is OXA-48 (and derivatives), which was first reported in a Turkish MDR *K. pneumoniae* isolate in Paris, France (25). OXA-48 efficiently hydrolyzes narrow-spectrum β-lactams such as penicillins, weakly hydrolyzes carbapenems, and spares broad-spectrum cephalosporins (26). It has been found in all of the members of the family *Enterobacteriaceae*; however, it is found mostly in isolates of *K. pneumoniae* (mostly of nosocomial origin) and *Escherichia coli* (mostly of community origin). OXA-48-producing *K. pneumoniae* is endemic in Turkey and certain North African and European countries (e.g., Morocco, Tunisia, Spain, Belgium) and shows a wide range of susceptibility profiles (25). Indeed, the MICs of carbapenems may vary significantly from isolate to isolate, depending on the host permeability background. Similarly, susceptibilities to broad-spectrum cephalosporins can also vary significantly, depending on the coproduction of other β-lactamases such as the ESBLs. Some OXA-48 derivatives, i.e., OXA-181, OXA-204, and OXA-232, all with similar hydrolytic properties, have also been identified in *K. pneumoniae* (27). These enzymes have been identified in North Africa, Australia, and New Zealand, but one of the main sources of OXA-181 (which is the second most common OXA-48 derivative) is the Indian subcontinent. Finally, a different isoenzyme of OXA-48 named OXA-163, differing by a single amino acid substitution combined with four amino acid deletions, has been identified in Argentina (28). This variant shows specific hydrolytic fea-

tures since it strongly hydrolyzes broad-spectrum cephalosporins and has weak activity against carbapenems.

Carbapenemases possess variable hydrolytic activities, with the MBLs and KPC enzymes hydrolyzing carbapenems more efficiently than OXA-48-like enzymes. However, high-level carbapenem resistance among *K. pneumoniae* isolates with carbapenemases requires additional permeability deficiencies, regardless of the type of carbapenemase produced (24). Conversely, isolates of *K. pneumoniae* with all types of carbapenemases exhibiting low carbapenem MICs have been identified. This might explain the initial successful spread of *K. pneumoniae* with bla_{KPC} in the United States during the 1990s and the initial spread of *K. pneumoniae* with bla_{VIM} in Greece (some isolates with VIM-type enzymes have imipenem MICs lower than 0.5 μg/ml) (29). It is possible that the initial spread of *K. pneumoniae* with carbapenemases was due to isolates with low carbapenem MICs without permeability modifications.

GENETIC SUPPORT OF CARBAPENEMASE GENES

The different carbapenemase genes circulating within *K. pneumoniae* are often carried by mobile structures, including plasmids and transposons, and therefore can spread efficiently to different members of the family *Enterobacteriaceae*. Transposon Tn*4401* has been shown to be the main genetic structure enhancing the spread of bla_{KPC}-type genes onto different plasmid scaffolds, but its transposition is not very efficient and the frequency of transmission has been quantified at 4.4×10^{-6} (30, 31). Tn*4401* is 10 kb in length, is delimited by two 39-bp imperfect inverted repeat sequences, and contains a Tn*3* transposase gene, a Tn*3* resolvase gene, and two insertion sequences, IS*Kpn6* and IS*Kpn7*. The association of Tn*4401*with bla_{KPC} and other antibiotic resistance determinants provides an easy way for carbapenemases to effectively spread as hitchhiker genes, even in the absence of carbapenem selection (32).

The bla_{OXA-48} gene is located in the Tn*1999* composite transposon that was shown to transpose at a very low frequency ($<1.0 \times 10^{-7}$) (33). The current dissemination of bla_{OXA-48} is therefore due mainly to the epidemic IncL/M-type plasmid (pOXA-48a) that was shown to be highly transferable (34, 35). The MBL genes (e.g., bla_{IMP}, bla_{VIM}, and bla_{NDM}) are found on different broad-host-range plasmid types (e.g., IncA/C, IncN) with various different genetic features (36); bla_{IMP} and bla_{VIM} are usually found in class 1 integron structures located within transposon structures that enhance their dissemination. Conversely, the bla_{NDM} genes are associated with mosaic genetic structures, including insertion sequences (e.g., IS*Aba1*), but the exact mechanism leading to their acquisition on plasmid scaffolds remains unknown (37).

HIGH-RISK CLONES AMONG OTHER CARBAPENEMASE PRODUCERS

High-risk clones are defined as clones with a global distribution that show an enhanced ability to colonize, spread, and persist in a variety of niches (38). High-risk clones have acquired certain adaptive traits that increase their pathogenicity and survival skills accompanied by the acquisition of antibiotic resistance determinants. They have the tenacity and flexibility to accumulate and exchange resistance and virulence genes with other bacteria. High-risk clones have contributed to the spread of different plasmids, genetic platforms, and resistance genes among Gram-negative bacteria and have played a very important role in the global spread of antibiotic resistance (39). Such clones are a powerful source for the propagation of genetic components of antimicrobial resistance (i.e., genes, integrons, transposons, and plasmids) (39). Drug resistance determinants are provided to offspring in a vertical fashion, and such eminent or high-risk clones increase the prevalence of antibiotic resistance by enhancing the abilities to survive and reproduce efficiently. The habitat of *K. pneumoniae* is not limited to humans but extends to the ecological environment, which includes surface water, sewage, and soil (2). Moreover, because of the ability of some isolates, including *K. pneumoniae* strains with carbapenemases (e.g., bla_{KPC} and bla_{NDM}), to survive for long periods of time in the environment at extreme temperatures, they play import roles in the horizontal transfer of drug resistance determinants to other bacteria, acting as efficient donors and recipients (40, 41).

CC258: ST258

The rate at which carbapenem resistance has disseminated globally in *K. pneumoniae* is cause for alarm in the medical community at large. To date, bla_{KPC} has been found in more than 100 different STs, but this pandemic is driven primarily by the spread of KPC-producing *K. pneumoniae* isolates that are members of clonal complex 258 (CC258) (18). CC258 (the founder member is ST292) consists of one predominant ST, namely, ST258, and to a lesser extent ST11, ST340, and ST512, which are single-locus variants of ST258 (18, 32). *K. pneumoniae* ST258 is a prototype of a high-risk clone, and recent information about the epidemiology, genetic rearrangement, and evolution of this successful clone has provided insights into the global spread of antimicrobial drug resistance.

K. pneumoniae with bla_{KPC} was first identified in a non-ST258 isolate in 1996 in the southern United States (42). In the late 1990s to the early 2000s, there were sporadic reports of *K. pneumoniae* with bla_{KPC} in the northeastern United States; however, large outbreaks due to related isolates were not described (43). In 2009, the Centers for Disease and Prevention in the United States, in collaboration with investigators from Israel, performed multilocus sequence typing of *K. pneumoniae* with bla_{KPC} and identified ST258 among the isolates collected in the New York area in 2005 (44). As time progressed, ST258 was detected in geographically diverse regions of the United States, and in 2009, it became apparent that ST258 was the predominant clone in the country, being responsible for 70% of the *K. pneumoniae* isolates with bla_{KPC} obtained from different parts of the country (45). During the mid-2000s, Israel experienced several nosocomial outbreaks of infections due to *K. pneumoniae* with bla_{KPC} caused by a clone (identified by pulsed-field gel electrophoresis) named clone Q (44). Interestingly, clone Q has a pulsotype similar to that of ST258 present in the United States. This was followed by global reports of ST258 among *K. pneumoniae* isolates with bla_{KPC} in countries such Greece (46), Norway, Sweden (47), Italy (48), Poland (49), Canada (50), Brazil (51), and Korea (52), suggesting that this ST has characteristics of international high-risk MDR clones. Recent reports from Israel (53) and Italy (54) demonstrated the endemicity and persistence of CC258 over time while it remained the predominant clone among *K. pneumoniae* isolates with bla_{KPC}. Interestingly, Israel has seen a dramatic overall decrease in the incidence of KPC enzymes among *K. pneumoniae* isolates, but ST258 still remains the predominant clone (53).

Kreiswirth and colleagues recently performed whole-genome sequencing of two *K. pneumoniae* ST258 urinary isolates from New Jersey and then did supplementary sequencing of a different global collection of just over 80 CC258 clinical isolates (55). The phylogenetic single nucleotide polymorphism (SNP) analysis of the core genomes of these isolates showed that *K. pneumoniae* ST258 belonged to two well-defined lineages named clades I and II. Clade I was associated with KPC-2, and clade II was associated with KPC-3. The genetic divergence of these two clades occurred in a 215-kb area that included the genetic material used for capsule polysaccharide biosynthesis (*cps*), an important virulence factor for *K. pneumoniae*.

The same group then compared the genetic structures of the *cps* regions and distribution of SNPs in the core genomes of ST258 clades I and II with those of other *K. pneumoniae* STs (i.e., ST11, ST442, and ST42) (56). Kreiswirth and colleagues found a 1.1-Mbp area in ST258 clade II that is identical to that of ST442, while the remainder of the ST258 genome was homologous to that of ST11. This indicates that ST258 clade II is a hybrid or crossbreed clone that was created by a large recombination event between ST11 and ST442. The investigators then identified the same *cps* regions in ST42 and ST258 clade I. The likeness of the areas surrounding the *cps* regions from ST42 and ST258 clades I and II indicated that ST258 clade I evolved from ST258 clade II because of the replacement of the *cps* region from ST42.

CC258: OTHER SEQUENCE TYPES

ST11, which is closely related to ST258, is the major ST among *K. pneumoniae* strains harboring bla_{KPC} from Asia (especially China) (57), has also been described in Latin America (18) and has been associated with NDM-type enzymes (58, 59) from the Czech Republic (60), Switzerland (61), Thailand (62), Australia (63), the United States (64), the United Arab Emirates (65), and Greece (66), being responsible for nosocomial outbreaks in the latter two countries. ST11 with bla_{OXA-48} has recently been identified in Spain (67). Other STs also belonging to CC258 with bla_{KPC} have been reported in Colombia (ST512), Italy (ST512), Israel (ST512), Spain (ST512), Brazil (ST340), and Greece (ST340) (18, 68).

OTHER SEQUENCE TYPES

K. pneumoniae ST147 is an emerging high-risk clone that was first identified in Greece (69) and has been associated with bla_{VIM} and bla_{KPC} in that country (46). This global ST has also been associated with bla_{NDM} (70) and $bla_{OXA-181}$ (71) in various countries, including Switzerland, Iraq, Canada, the United Kingdom, India, and Italy. ST14, ST25, and ST340 with bla_{NDM-1} have been identified in India, Kenya, and Oman (72), and ST405 with OXA-48 has been identified in Spain (67).

THE IMPORTANCE OF EPIDEMIC PLASMIDS IN THE SPREAD OF CARBAPENEMASE GENES

Plasmids are extrachromosomal elements of double-stranded DNA present in bacteria that replicate independently of the host genome (73). Plasmids can undergo horizontal transfer through conjugation, thereby transferring the encoded genetic elements from one bacterium to another. This movement of plasmid-borne antibiotic resistance genes has been central to the recent and rapid increase in global antimicrobial resistance (17). DNA on plasmids used for replication purposes needs to be conserved and therefore is utilized for the classification of plasmids. This "incompatibility group typing" scheme is based on unique replication areas identified in different plasmids to demonstrate the relatedness and behavior of particular plasmid groups (74, 75). Antimicrobial resistance plasmids can be broadly divided into two main groups, namely, the narrow-host-range group that most often belongs to incompatibility (Inc) group F and the broad-host-range group that belongs to IncA/C and IncN. They have recently been termed "epidemic resistance plasmids" because of their propensity to acquire resistance genes and rapid dissemination among members of the family *Enterobacteriaceae* (76). Antimicrobial resistance determinants on epidemic plasmids provide a selective advantage to high-risk clones and are likely central to their success (77, 78).

PLASMIDS ASSOCIATED WITH *K. PNEUMONIAE* ST258 WITH bla_{KPC}

Several different KPC-containing plasmids have been identified in ST258. They belong to IncF (with FII_{K1}, FII_{K2}, and FIA replicons), IncI2, IncX, IncA/C, IncR, and ColE1, and these plasmids often contain various genes encoding nonsusceptibility to different antimicrobial drugs (32). However, the predominant bla_{KPC} plasmid type associated with *K. pneumoniae* ST258 is IncF with FII_K replicons (79). The first bla_{KPC} plasmid identified in ST258 (named clone Q at that time) was obtained in 2006 in Israel and named pKpQIL (80). This was a 113-kb IncF plasmid with an FII_{K2} replicon containing Tn*4401a* and a backbone very similar to that of the pKPN4 plasmid first characterized in 1994 from non-KPC antimicrobial-resistant *K. pneumoniae* obtained in Massachusetts (80).

Retrospective plasmid analysis of *K. pneumoniae* with bla_{KPC} isolated during the early 2000s in the New York and New Jersey areas showed that ST258 contained bla_{KPC-2} and bla_{KPC-3} on pKpQIL-like plasmids that were nearly identical to the Israeli pKpQIL plasmid described in 2006 (81). The pKpQIL-like plasmids from the New York and New Jersey isolates were associated mostly with bla_{KPC-2} and to a lesser extent with bla_{KPC-3}, whereas the pKpQIL plasmids from Israel were associated mainly with bla_{KPC-3} (81, 82). This suggests that ST258 with bla_{KPC-3} on pKpQIL plasmids was introduced during the mid-2000s from the United States into Israel (a founder effect), followed by clonal expansion in Israel. The $IncFII_K$ plasmids are also the most common plasmids identified in ST258 with bla_{KPC} in several different geographically diverse areas, including Canada, Poland, the United States, Israel, Brazil, Italy, and Norway (51, 79, 83). There also appears to be an association between different plasmid Inc groups and ST258 clades I and II. The bla_{KPC-3}-associated IncI2 plasmids and bla_{KPC-3}-associated IncFIA plasmids were found exclusively in clade II, while the pKpQIL-associated $IncFII_{K2}$ plasmids with bla_{KPC-2} were detected in both clades I and II (55). pKpQIL plasmids were not only restricted to ST258 but also present in 33% of the non-ST258 *K. pneumoniae* isolates in the New York area (81).

The complete sequences of plasmids associated with ST258 from large collections reveal that they are evolving over time through large genetic rearrangements (79, 84, 85). This process is creating hybrid plasmids, as was previously described in Italy, with ST258 containing two different IncF plasmids, namely, pKpQIL-IT and pKPN-IT, as well as a ColE1-like plasmid with bla_{KPC-2} (86). Both pKpQIL-IT and pKPN-IT have a very high degree of homology to historic plasmids pKPN4 and pKPN3 from a non-KPC-producing *K. pneumoniae* strain isolated in 1994 (86).

This suggests that certain ancestral plasmids are particularly well suited to *Enterobacteriaceae*, such as *K. pneumoniae*, and are good candidates for sustaining the presence of bla_{KPC} through multiple independent insertion and transposition events. This is further supported by a recent study in Korea that demonstrated that ancestral plasmids were present among the ST258 isolates found in various geographical regions and were obtained as early as 2002 (87).

It seems that the presence of plasmids with bla_{KPC} is central to the success of ST258. The loss of pKpQIL by ST258 has limited the ability of these isolates to successfully disseminate compared to that of other *K. pneumoniae* isolates without bla_{KPC} and ST258 with pKpQIL (78). This suggests that bla_{KPC}, in combination with other virulence or persistence factors on the pKpQIL-like plasmids, promoted the fitness and survival of ST258. This is further supported by the epidemiological observation that non-ST258 *K. pneumoniae* with bla_{KPC} did not demonstrate the same global success as ST258 with bla_{KPC}. It appears that the successful global dissemination and survival of *K. pneumoniae* ST258 are in part dependent on the combination of bla_{KPC} on IncF plasmids with factors inherently present on the chromosome of this high-risk clone (77).

IncI2 with bla_{KPC-3} can also successfully pair with ST258 and was recently detected in 23% of the ST258 isolates obtained in the New York and New Jersey areas (88). Interestingly, this IncI2 plasmid also encoded type IV pili, which may contribute to the successful dissemination of *K. pneumoniae* ST258.

PLASMIDS ASSOCIATED WITH bla_{NDM} AND bla_{OXA-48}

The current global dissemination of NDM-1-producing *K. pneumoniae* is linked to the dissemination of epidemic broad-host-range plasmids. Several epidemiological studies showed a high diversity of plasmid backbones bearing the bla_{NDM} genes. Molecular epidemiology indicated that the IncA/C-type plasmids are the main backbones responsible for spreading bla_{NDM-1} among members of the family *Enterobacteriaceae* (72, 89), but IncFII, IncN, IncH, and IncL/M types have also been identified in association with bla_{NDM} (90–92). It is noteworthy that IncA/C plasmids with bla_{NDM} often contain various clinically relevant antibiotic resistance genes, such as those encoding RmtA and RmtC (16S rRNA methylases encoding high-level resistance to aminoglycosides), QnrA (quinolone resistance), and CMY-type β-lactamases (broad-spectrum cephalosporin resistance).

In contrast to what has been observed with bla_{NDM} genes, the current emergence of OXA-48-producing isolates in many geographical areas is explained mainly by the success of one specific plasmid (pOXA-48a). This plasmid is 62 kb in size and belongs to the IncL/M group (34). It is noteworthy that it possesses bla_{OXA-48} as a unique antibiotic resistance gene, in contrast again with bla_{NDM}-positive plasmids, which often contain several antibiotic resistance genes. Plasmid pOXA-48a is self-conjugative, and it has been demonstrated that its *tir* gene, known to encode a transfer inhibition protein, was truncated. This inactivation was shown to be responsible for a 50- to 100-fold increase in the efficiency of transfer of pOXA-48a and therefore explains the very high conjugation rate of the latter plasmid, which was estimated to be around 1×10^{-1} (35). Therefore, it is considered that these specific features of plasmid pOXA-48a do explain, in large part, the current spread of the OXA-48-encoding gene.

THE SUCCESS AND VIRULENCE FACTORS OF *K. PNEUMONIAE* ST258

K. pneumoniae is responsible for human and animal infections and has also been implicated in diseases of certain plants, such as spinach, rice, and pineapples (93). It remains unclear how one bacterium is successful in causing infections in plants and humans. *K. pneumoniae* also has the ability to survive for long periods in the hospital environment (83). Recently, Lerner and colleagues identified superspreaders among carbapenemase-producing *K. pneumoniae* isolates from rectal and environmental specimens (94). These superspreaders were more likely to be present at high rectal concentrations and more likely to be present at high concentrations in the immediate environment, which may play a central role in the transmission of carbapenemase-producing *K. pneumoniae*. Reservoirs in patient or health care worker populations and the environment represent principal modes of spread in nosocomial outbreaks, with the patient population being the most important reservoir in high-frequency outbreaks (83, 94).

The global molecular epidemiology of KPC-producing bacteria shows that *K. pneumoniae* is the most common species and ST258 is the predominant clone, suggesting a unique fitness and selective advantage beyond merely antimicrobial resistance. The reasons for the particular success of ST258 and its association with certain resistance plasmids are uncertain. However, its ability to spread swiftly is beyond dispute.

It is unclear if ST258 has greater virulence than other *K. pneumoniae* isolates. A recent study demonstrated that ST258 is nonvirulent in animal models, is highly susceptible to serum killing, and rapidly undergoes phagocytosis (95). Another study showed that not all ST258 isolates behaved the same way in a mouse lethality model, but consistency did exist in a moth (*Galleria mellonella*) virulence model (96). ST258 also lacks well-characterized *K. pneumoniae* virulence factors, including K1, K2, and K5 capsular antigen genes, aerobactin genes, and the regulator of mucoid phenotype gene *rmpA* (95). Lavigne and colleagues, using the *Caenorhabditis elegans* model, have shown that the plasmid with bla_{KPC} is not necessarily associated with increased virulence (97).

Capsular polysaccharide is a recognized virulence factor that enables *K. pneumoniae* to evade phagocytosis. The in-depth molecular epidemiologic examination of the genome region from different clades of ST258 that have independently acquired bla_{KPC} revealed that capsule polysaccharide biosynthesis regions *cps-1* and *cps-2* are likely involved in the global success of these clades (56, 98). This region of diversification could be advantageous for *K. pneumoniae* isolates to change polysaccharide as a mechanism to evade host defenses. Capsule switching is a species-specific mechanism used by bacteria to escape the host immune response. DNA exchange in and around the *cps* regions may be an important mechanism used by *K. pneumoniae* to rapidly diversify and evolve (99).

Adler and colleagues investigated the association of the integrated conjugative element ICEKp258.2 with ST258 by testing 160 *K. pneumoniae* strains of diverse STs for the presence of *pilV*, a gene carried on ICEKp258.2 (100). They found that *pilV* was present only in ST258 and genetically related STs such as ST512. On the basis of sequence analysis, ICEKp258.2 harbors a type IV pilus gene cluster and a type III restriction-modification system. A type IV pilus could increase the uptake and exchange of DNA, such as

plasmids, as well as facilitate adherence to living and nonliving surfaces, which may in part explain the high transmissibility of ST258. Additionally, a type III restriction-modification system could serve in "host specificity" regarding the exchange of certain compatible plasmids and other mobile elements (56). The restriction of plasmids and specific mobile elements may explain the differences observed between ST11 (which lacks ICEKp258.2) and ST258, as the former is associated with a broad range of plasmids and carbapenemases (KPC, VIM, IMP, NDM, and OXA-48), whereas ST258 strains predominantly harbor KPC. Taken together, the association of ICEKp258.2 with *K. pneumoniae* ST258 strains raises the possibility that this element contributes to the epidemiological success of this ST (56).

So far, no specific virulence factor has been identified in those widespread clones producing NDM- or OXA-48-type enzymes, the main driving factor of those disseminated clones apparently being resistance to antibiotics only.

TREATMENT OF INFECTIONS DUE TO *K. PNEUMONIAE* WITH CARBAPENEMASES

Infections due to *K. pneumoniae* with carbapenemases often reach mortality rates ranging between 23 and 75%, which are attributed to the lack of active antimicrobial agents and underlying comorbidities of patients (101). A delay in the appropriate antibiotic therapy for severe infections is strongly associated with impaired outcomes and increased mortality rates for patients with severe sepsis and septic shock and is also relevant for patients with infections due to *K. pneumoniae* with carbapenemases (101). The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* is unknown, and none of the currently available antibiotics used as single therapy may be effective for treating infections with all types of carbapenemase producers. Source control, in addition to antimicrobial therapy, is essential for the effective management of these infections and is especially significant for the successful treatment of UTIs and intra-abdominal infections. Empirical combination therapy including colistin, a carbapenem, or an aminoglycoside, based on the local resistance epidemiology, might be justified for severely ill patients with suspected infections due to *K. pneumoniae* strains with carbapenemases (102).

Most clinical data on the efficacy of antibiotics for treating carbapenemase producers are from retrospective case series and anecdotal case reports and mostly involve KPC-producing *K. pneumoniae* (103, 104). It seems logical to tailor antimicrobial therapy to the *in vitro* patterns of microbial susceptibility to tested antibiotic molecules, and definitive therapy should always be guided by susceptibility testing. Often, polymyxins (e.g., colistin or polymyxin B), tigecycline, fosfomycin, and sometimes selected aminoglycosides are the only agents with *in vitro* activity. Other antimicrobials, such as fosfomycin and nitrofurantoin, can be used if found to be active, but their use as monotherapy is generally limited to lower UTIs (102). Since carbapenemase producers are mostly resistant to various other important antibiotic classes, such as fluoroquinolones and aminoglycosides, it is important to test for susceptibility to last-resort agents such as polymyxins (e.g., colistin), fosfomycin, tigecycline, and rifampin.

Patterns of susceptibility to antibiotics, in particular, β-lactam drugs, depend on the carbapenemase type. KPC producers are usually resistant to all β-lactams; however, temocillin does retain activity against some isolates and this drug is a treatment option for lower UTIs due to *K. pneumoniae* with bla_{KPC} (105). NDM, VIM, and IMP producers remain susceptible to aztreonam, while OXA-48-like producers may test susceptible to the expanded-spectrum cephalosporins in approximately 20% of the cases (14). Combined mechanisms of resistance to β-lactams are often observed among carbapenemase-producing *K. pneumoniae* strains (22, 25, 37).

Combined therapy may maximize bacterial killing (synergistic effect) and minimize bacterial resistance. The best antibiotic associations contain two molecules that show *in vitro* activities against carbapenemase producers (103, 106). Several studies have indicated that the mortality rate was significantly lower in patients given combination therapy (106, 107), while other studies have indicated that the superiority of combined therapy to monotherapy was not significant (103). A recent review article recommended using combination therapy to treat bloodstream infections when MDR bacteria are suspected (108). Doi and Paterson, on the basis of an extended analysis of *in vivo* efficacy data, recommended combination therapy that includes a carbapenem with a second agent such as colistin, tigecycline, or gentamicin, depending of the results of *in vitro* susceptibility testing (109).

Options for the treatment of infections with carbapenemase-producing *K. pneumoniae* are limited. Some studies suggest that for infections due to KPC producers, the use of combination therapy that includes a carbapenem (e.g., polymyxin-carbapenem or aminoglycoside-carbapenem), may reduce the mortality rate (101). Clinical data on the treatment of infections due to OXA-48 and NDM infections are scant; a recent retrospective observational study suggested that for bacteremia due to OXA-48 producers, combination therapy that included colistin reduced the mortality rate (110).

Colistin (polymyxin E) was discovered more than 60 years ago, while polymyxin B is available in only a limited number of countries. The major side effect of these molecules is nephrotoxicity, while the optimal dosage is unknown. Colistin has become the most popular agent for the treatment of infections due to *K. pneumoniae* with carbapenemases (101, 102). Colistin monotherapy has been associated with mortality rates exceeding 50% when used for severe infections (111), and one Brazilian study showed that combination therapy was not superior to monotherapy (112). Recent understanding of the pharmacokinetics of colistin has resulted in the use of doses higher than those used in early studies. The current recommendations include a loading dose and a total standard dose of 9 to 10 million international units daily divided into two or three doses (113). This molecule has significant activity against various carbapenemase-producing isolates and is often used in combination therapy (e.g., with aminoglycosides, aztreonam, carbapenems, rifampin, tigecycline, or fosfomycin) (103, 104). Unfortunately, because of the increased use of this agent, colistin-resistant *K pneumoniae* isolates are increasingly being reported (114).

Intravenous fosfomycin is available in Europe, where it has been used in combination with tigecycline and colistin to treat severe infections due to MDR bacteria (115). *in vitro* analysis indicated synergistic activity of colistin and fosfomycin against some NDM producers (116).

Tigecycline is a tetracycline derivative and has been available since 2005. This molecule does not diffuse sufficiently into the urinary tract, where many infections due to carbapenemase-producing *K. pneumoniae* originate (104). In 2013, the FDA issued a warning indicating an increased rate of death when tigecycline is

used (2.5%) rather than other antibiotics (1.8%) that were related to treatment failures (104). In addition, acquired tigecycline resistance has been reported in patients infected with KPC-producing *K. pneumoniae* (117). A recent report suggested that high-dose tigecycline (100 mg every 12 h following a 200-mg loading dose) may provide better outcomes than conventional doses do (118).

Rifampin has a very broad spectrum of activity that includes the family *Enterobacteriaceae*. Several reports show some *in vitro* synergy in the killing of carbapenemase-producing *K. pneumoniae* between rifampin and tigecycline or colistin (119, 120). However, definitive clinical data are lacking that advocate the routine use of rifampin for the treatment of infections due to carbapenemase-producing *K. pneumoniae*.

Several aminoglycoside molecules may retain activity against carbapenemase-producing *K. pneumoniae*. Some KPC and OXA-48 producers remain susceptible to gentamicin, while this is rare for NDM producers (121). Aminoglycosides have been used with some clinical success either alone or in combination therapy to treat infections due to KPC producers (106). A recent report suggested better outcomes when gentamicin (as monotherapy or in combination with tigecycline) was used for colistin-resistant, KPC-producing *K. pneumoniae* (122). The side effects of aminoglycosides include nephrotoxicity, especially when they are used in combination with colistin.

Carbapenems, despite being hydrolyzed by carbapenemases (hence the definition of those enzymes) may retain some activity against carbapenemase-producing *K. pneumoniae* (106, 123). Treatment regimens using carbapenems may be an option when the MICs of carbapenems are ≤8 mg/liter when a second antibiotic is added or when a prolonged intravenous infusion regimen is used (123, 124). Encouraging results have been obtained with VIM and OXA-48 producers in humans and with NDM producers in animal models (106, 125, 126). Studies performed with an animal model of infection (i.e., mouse pneumonia) suggested that dual-carbapenem therapy (i.e., meropenem plus ertapenem) may be effective (126). Ertapenem most likely acts as a "suicide" molecule for carbapenemase activity, whereas the more active drug, meropenem, retains its efficacy. Efficacy of this double-carbapenem therapy has been shown in humans infected with KPC producers (127). Among other β-lactams, the extended-spectrum (i.e., third- and fourth-generation) cephalosporins may be effective against OXA-48 producers without ESBLs (128), while aztreonam remains an option for treating infections due to MBL producers that test susceptible to this agent (37).

Several antibiotics in development may have significant activity against carbapenemase-producing *K. pneumoniae* (104). One of the most promising drugs is the combination of avibactam with ceftazidime. Avibactam is an efficient β-lactam inhibitor that inhibits the *in vitro* activity of serine β-lactamases such as KPC and OXA-48. Ongoing phase III studies show the efficacy of this inhibitor combination against KPC producers (104). Combinations of avibactam with other agents such as ceftaroline and aztreonam are in the developmental stages (phase I and II studies) (104). The advantage of the combination of avibactam and aztreonam would be in the treatment of infections due to isolates with MBLs (129). Another potent serine β-lactamase inhibitor is MK7655 in combination with imipenem, which sufficiently inhibits various KPC producers (104). Some promising molecules include the aminoglycoside plazomicin (ACHN-490), which has significant activity against all types of carbapenemase producers except NDM producers, which often produce 16S rRNA methylases conferring resistance to all aminoglycoside molecules (130); the tetracycline analogue eravacycline for the treatment of KPC producers (104); and the novel polymyxins under development, such as NAB739, NAB4061, and NAB741, with lower nephrotoxicity (131).

Within the next 24 months, it is likely that the combination of avibactam and ceftazidime will be available in clinical medicine and may represent an important additional value for the treatment of the increasing number of difficult-to-treat infections due to carbapenemase producers. Implementation of hygiene measures, rapid detection of carbapenemase producers, and the use of the combination of avibactam and ceftazidime might be the cornerstones of the treatment and control of infections due to *K. pneumoniae* with KPC enzymes or OXA-48. However, the efficient treatment of MBL producers (i.e., VIM, IMP, and NDM) remains to be determined.

RECENT RECOMMENDATIONS

Rodríguez-Baño and colleagues in Spain (102) and Karaiskos and Giamarellou (101) in Greece recently published excellent recommendations regarding the treatment of infections with carbapenemase-producing *Enterobacteriaceae*. These recommendations or guidelines contain pertinent and detailed information on this important topic, and we urge interested readers to scrutinize those articles.

SUMMARY

The management of infections due to *K. pneumoniae* has been complicated by the emergence of antimicrobial resistance. Of special concern is the emerging resistance to carbapenems, since these agents are often the last line of effective therapy available for the treatment of infections caused by MDR *K. pneumoniae*. Resistance to carbapenems in *K. pneumoniae* may be linked to different mechanisms, and the co-occurrence of permeability defects together with the production of certain β-lactamases (e.g., AmpC cephalosporinases) possessing very weak carbapenemase activity may lead to reduced susceptibility to carbapenems. True carbapenemases are responsible for nonsusceptibility to carbapenems without additional permeability defects in *K. pneumoniae*. Those carbapenemases belong to Ambler molecular class A (i.e., KPC, GES), B (i.e., NDM, VIM, IMP), or D (i.e., OXA-48-like). A summary of the classification, spectrum of activity, inhibition properties, types, regions of endemicity, and molecular epidemiology of carbapenemases in *K. pneumoniae* is shown in Table 1.

K. pneumoniae ST258 is an important human pathogen, has spread extensively throughout the world, and is responsible for the rapid increase in the prevalence of antimicrobial-resistant *K. pneumoniae*. This clone is known to cause UTIs, respiratory tract infections, and BSIs and is associated with carbapenemase production, most often KPC-2 and KPC-3. Recent molecular studies have shown that ST258 consists of two distinct lineages, namely, clades I and II. Clade I is specifically associated with KPC-2, and clade II is specifically associated with KPC-3. The genetic differentiation between the two clades resulted from a 215-kb region of divergence that includes genes involved in capsule polysaccharide biosynthesis, indicating that these two clades have followed distinct evolutionary pathways. Additional investigation showed that ST258 clade II is a hybrid clone that was created by a recombination event between ST11 and ST442. Moreover, it seems that

ST258 clade I strains evolved from a clade II strain as a result of replacement of the *cps* region from ST42.

The integrative conjugative element ICEKp258.2 contains gene clusters for a type IV pilus (i.e., *pilV*) and a type III restriction-modification system. *pilV* on ICEKp258.2 may be responsible in part for the high transmissibility and durability of ST258 on foreign surfaces, and it seems that this integrative conjugative element contributes significantly to the epidemiological success of *K. pneumoniae* ST258. Different KPC-encoding plasmids have been identified in ST258, and $IncFII_{K1}$ and FII_{K2} are the most common. These plasmids often contain several genes encoding resistance to other antimicrobial agents, such as aminoglycosides, quinolones, trimethoprim, sulfonamides, and tetracyclines, and have played an important role in the success of ST258.

The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* is unknown, and none of the currently available antibiotics used as single therapy may be efficient for the treatment of all types of carbapenemase producers. Various agents, such as polymyxins, fosfomycin, tigecycline, rifampin, and carbapenems, most often as part of combination therapy, have been used with various degrees of success to treat infections due to MDR *K. pneumoniae*. Several antibiotics in development (e.g., avibactam with ceftazidime) have significant activity against carbapenemase-producing *K. pneumoniae*, especially those with KPC enzymes. However, effective options for the treatment of infections due to NDM producers remain elusive.

Infection control measures that have been shown to be effective in successfully decreasing the acquisition of carbapenemase-producing *K. pneumoniae* include combined interventions of increased compliance with hand hygiene, contact precautions, environmental cleaning, early identification of asymptomatic carriers, and the physical separation of carbapenemase-producing *K. pneumoniae*-positive patients and their staff (132). Prompt and appropriate infection control measures should be implemented upon the isolation of carbapenemase-producing *K. pneumoniae*. Expert guidelines on infection control measures have been provided by the Centers for Disease Control and Prevention and the European Society of Clinical Microbiology and Infectious Diseases (133). Colonized or infected patients should be isolated individually or in groups and treated in accordance with strict infection control directives, including hand disinfection, the use of gowns and disposable aprons, and proper cleaning (111).

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